Table 2. Histamine released from normal cell suspensions after 4 hours' incubation with allergic serum (pt. *Bo.*) and 1 hour's incubation with Timothy antigen (0.3 μg protein nitrogen per milliliter). Each sample was suspended in 3 ml of serum and 2 ml of Tyrode's solution.

Cell samples	Incubation		Histamine (μg/liter)
	4 hours	1 hour	
1–2	Serum	Antigen	17.8
3–4	Serum	No antigen	2.3
5–6	Serum*	Antigen	2.1
7–8	Serum†	Antigen	20.6
9–10	Serum*†	Antigen	2.1

* Serum was heated at 56°C for 30 min. † Cells suspended in 2 ml of allergic serum, 1 ml of fresh unheated normal serum, and 2 ml of Tyrode's solution.

serum did not restore the activity of heated allergic serum (Table 2).

Generally, we removed the allergic serum from the cells before adding antigen; this was done to reduce fluorescence contributed by serum factors (including histamine). However, histamine release after the addition of antigen was similar when the serum was left in the system.

In the first series of studies made possible by establishing the conditions essential for passive transfer in vitro, we measured the rate of sensitization of normal cells by various dilutions of allergic serum. In Fig. 1 are the results of experiments with three

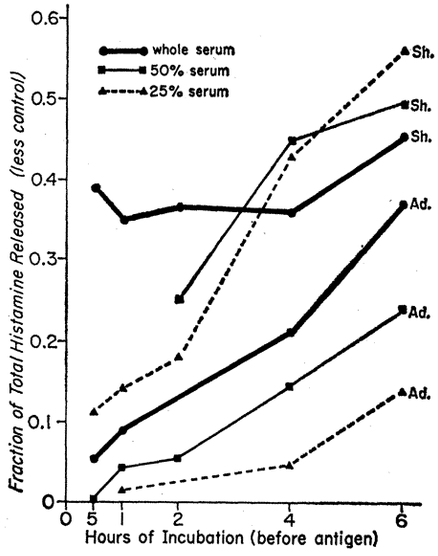

Fig. 1. Histamine released from *Va.* cells by Timothy antigen (.03 μg protein nitrogen/ml) after incubation with sera from donors *Sh.* and *Ad.* Reaction volume was 2 ml. Serum was removed and replaced with Tyrode's solution before antigen was added. Since the actual concentration of histamine released by antigen was directly proportional to the total available cellular histamine for any experimental condition, the results are expressed as the fraction of total histamine released.

dilutions of two allergic sera. The cells were from the same donor, and both the reaction volumes and cell concentration were kept constant. In each experiment, the serum was removed at the end of the first incubation and the cells were resuspended in Tyrode's solution before antigen was added. The controls, for each point, consisted of the identical steps, but with no antigen in the last hour of incubation. It is apparent that these sera differ, both in the rate of sensitization, and in the maximum amount of histamine that can be released. When serum *Sh* was diluted, the rate of sensitization was slower, yet the degree of sensitization after 4 and 6 hours incubation was greater than with whole serum. The enhancing effect of dilution has occurred consistently in repeated studies with three sera (including *Sh*); the differences are not great enough, however, to suggest the presence of a serum inhibitor. By contrast, two other sera (including *Ad*, Fig. 1) sensitized the cells at a slower rate, and histamine release was roughly proportional to the assumed antibody concentration. Fortunately, the spontaneous release of histamine during serum incubation up to 6 hours has always been relatively small so that the available histamine for antigenic release is not appreciably reduced.

The major difference between these studies and earlier ones which produced unsatisfactory results, both in our laboratory and elsewhere, was the recognition that the allergic sera were unexpectedly labile during handling and storage. Although the skin-sensitizing antibody is known to be more labile than other antibodies, its activity (as measured by passive transfer to skin) is abolished only by prolonged heating (up to 4 hours), and storage, even at room temperature, has no measurable effect for as long as 6 months (*6*).

Histamine release caused by specific antigen is an indicator of specific reactivity which has both qualitative and quantitative implications for studying human allergy. If attention is given to maintaining the activity of allergic sera, passive transfer of reactivity to other human tissues such as lung should be feasible, and precision should improve as purified antigens become available (*7; 8*).

PAUL P. VANARSDEL, JR.
C. J. SELLS*
Department of Medicine,
School of Medicine,
University of Washington, Seattle 5

References and Notes

1. G. Katz and S. Cohen, *J. Am. Med. Assoc.* **117**, 1782 (1941); J. W. Noah and A. Brand, *J. Allergy* **25**, 210 (1954); P. P. VanArsdel, Jr., *et al., ibid.* **29**, 429 (1958).
2. P. P. VanArsdel, Jr., *J. Clin. Invest.* **41**, 1407 (1962).
3. V. V. Chambers, B. H. Hudson, J. Glaser, *J. Allergy* **29**, 93 (1958).
4. E. Middleton, Jr., *Proc. Soc. Exptl. Biol. Med.* **104**, 245 (1960).
5. P. A. Shore, A. Burkhalter, V. H. Cohn, Jr., *J. Pharmacol. Exptl. Therap.* **127**, 182 (1959); J. W. Noah and A. Brand, *J. Allergy* **31**, 3 (1961); L. T. Kremzner and I. B. Wilson, *Biochim. Biophys. Acta* **50**, 364 (1961).
6. J. G. Harter, *Federation Proc.* **20**, 259 (1961).
7. L. M. Lichtenstein and A. G. Osler, *ibid.* **22**, 560 (1963).
8. Supported by grant AI 01510-07 from the National Institutes of Health. We thank Mrs. Deidre Tucker for valuable technical assistance.
* Student summer research fellow, Allergy Foundation of America, 1961 and 1962.

14 June 1963

Carbohydrate Digestion in the Cockroach

Abstract. *The digestive tract of the cockroach,* Blaberus craniifer *Burmeister, contains amylase, α- and β-glucosidases, β-fructofuranosidase, α- and β-galactosidases, but no cellulase. The most active site of hydrolytic action is the midgut, followed by the foregut, hindgut, salivary gland tissue, and Malpighian tubules.*

The digestive enzymes of cockroaches have received the attention of many investigators (*1, 2, 3*). It is desirable to broaden the base for generalization regarding the distribution of carbohydrases in this group of insects and to have specific information about additional cockroach species. Accordingly, salivary glands, digestive tracts and Malpighian tubules of adult specimens of *Blaberus craniifer* Burmeister have been examined qualitatively for evidence of carbohydrase activity.

Cockroaches were reared in aquaria at about 26°C. Pablum (*4*) was provided twice a week, this being fortified periodically with Brewer's yeast. Prior to experimentation, the cockroaches were isolated and food was withheld for a minimum of 1 week, to permit their digestive tracts to be cleared of nutriment. Water was supplied continuously, but 24 hours before testing tap water was replaced by distilled water. During the final 12 hours, complete starvation was imposed.

The cockroaches were anesthetized with ethyl ether and their alimentary tracts removed. These were freed of adhering tissues and sectioned into fore-, mid-, and hindgut, and Malpighian tubules. Salivary glands were also re-

Table 1. Results of tests for carbohydrase activity in the digestive tract of *B. craniifer*.

Substrate	Salivary glands	Foregut	Midgut	Malpighian tubules	Hindgut	Type of enzyme
Glycogen	+	+	+		−	Amylase
Starch	+	+	+		−	Amylase
Cellulose	−	−	−		−	
Melezitose	−	−	+	−	−	α—Glucosidase
Raffinose		+	+	+	+	β—Fructofuranosidase
Sucrose		+	+	+	+	α—Glucosidase or β—Fructofuranosidase
Methyl glucoside		−	+	+	+	α—Glucosidase
Cellobiose	−	+	+	−	+	β—Glucosidase
Lactose	+	+	+	−	+	β—Galactosidase
Maltose	+	+	+	+	+	α—Glucosidase
Melibiose		+	+	−	+	α—Galactosidase
Control No. 1	+	+	+	−	+	β—Fructofuranosidase
Control No. 2	−	−	−	−	−	

moved and cleared of surrounding tissues. Like tissues from five cockroaches were pooled, and ground with clean white sand in a small amount of saline (0.9 percent NaCl, 0.02 percent KCl, 0.02 percent $CaCl_2$, 0.4 percent glucose). The resulting brei was filtered into test tubes, each suspension then being made up to 6.0 ml by the addition of saline.

To test for the presence of carbohydrases, experiments were conducted in vitro with substrates consisting of a di-, tri-, or polysaccharide, or a glycoside. Reaction mixtures were put in incubation tubes, each of which contained 0.5 ml of suspension, 1.0 ml of 2-percent substrate, 1.0 ml phosphate buffer (0.05*M*) adjusted to *p*H 6.5, and 5 drops of toluene, a bacterial inhibitor. To serve as controls, one tube contained the complete reaction mixture, sucrose substrate, but no buffer: another contained the complete reaction mixture and sucrose substrate, but was inactivated by boiling for 3 minutes (Table 1, Nos. 1 and 2, respectively). All tubes were incubated 24 hours at 36°C. To test for cellulase activity, finely divided filter paper was used as the substrate and the incubation time extended to 72 hours. Benedict's reagent was used to test for hydrolysis of non-reducing carbohydrates, while the hydrolytic products of reducing disaccharides were detected by the osazone method.

The fates of carbohydrates in the alimentary tract of *B. craniifer* were followed by means of paper chromatography. Pairs of cockroaches were provided with cotton swabs soaked with a 20-percent solution of the desired substrate. One pair (controls) were provided with swabs dampened with tap water. The substrates were left with the cockroaches for 3 hours, after which the animals were anesthetized and opened. Ligatures were applied at the juncture of the fore- and midgut, and between the mid- and hindgut. Each section was then aspirated, after puncture with a fine glass capillary, and the intestinal juice thus collected was applied in drops, 1 inch apart, to a sheet of 9- by 11-inch Whatman No. 1 filter paper. Chromatograms were run by the ascending method. The solvent system was *n*-butanol, pyridine, and water (3:1:3 vol/vol). Benzidine trichloroacetic acid (*5*) was the developing reagent. Presence of the original substrate or of its hydrolytic products was determined by comparing their positions with those of reference sugars.

Results are summarized in Table 1. Results obtained with Benedict's solution indicated the presence of amylase which hydrolyzes starch and glycogen to maltose and glucose. An α-glucosidase, which cleaves methyl glucoside and melezitose, as well as β-fructofuranosidase, which splits sucrose and raffinose, were present. There was no evidence of a cellulase.

Positive osazone tests indicated the presence of α- and β-galactosidases and α- and β-glucosidases, which catalyze the hydrolysis of melibiose, lactose, maltose, and cellobiose, respectively. Results obtained with the buffer-free control suggest that the influence of the buffer was negligible. The negative test results in the control that was heated (No. 2, Table 1) show that any enzymes that may have been present were inactivated.

Chromatograms of aspirated intestinal juices yielded spots which support the above findings. Indeed, definite evidence of melibiase activity in the hindgut, of lactase in the fore and hindgut, and of cellobiase in all regions, was obtained only by this procedure. By contrast, results of osazone tests, in the instances cited, were often inconclusive.

The distribution of carbohydrases in *B. craniifer* resembles that reported for *B. discoidalis* and *Leucophaea maderea* (*3*). It differs from the latter two species but resembles most other insects in its apparent lack of cellulase (*2, 6*). The midgut is the site of the most vigorous enzymatic activity, there being a gradual decrease in activity in other parts of the tract. This is consistent with histological findings of a preponderance of secretory epithelium in the midgut, slight extension of secretory cells into the caeca, and none in the other areas. It seems reasonable, then, to ascribe the carbohydrase activity of the fore- and hindgut regions partly to overflow from the midgut, and partly to enzymes exuded from macerates of the two sections (*7*).

WILLIAM M. BANKS
Department of Zoology, Howard University, Washington 1, D.C.

References and Notes

1. R. L. Abbott, *J. Exptl. Zool.* **44**, 219 (1926); V. B. Wigglesworth, *Biochem. J.* **21**, 797 (1927); M. F. Day and R. F. Powning, *Australian J. Sci. Res. Ser. B* **2**, 175 (1949).
2. H. S. Swingle, *Ohio J. Sci.* **25**, 209 (1925).
3. P. Ehrhardt and G. Voss, *J. Insect. Physiol.* **8**, 165 (1962).
4. A concentrated baby food with high protein content produced by Gerber Products Co., Fremont, Mich.
5. J. S. D. Bacon and J. Edelman, *Biochem. J.* **48**, 114 (1951).
6. W. A. L. Evans, *Exptl. Parasitol.* **5**, 191 (1956); D. Gilmour, *The Biochemistry of Insects* (Academic Press, New York, 1961), pp. 40–59.
7. I thank M. S. Briscoe for supplying the adult specimens of *Blaberus craniifer* which were used in these experiments.

10 July 1963

cis-3-Chloroacrylic Acid: A New Cotton Defoliant and Crop Desiccant

Abstract. *cis-3-Chloroacrylic acid is a potent cotton defoliant and a crop desiccant. Relationships between structure and activity indicate a relatively high degree of specificity, since minor modifications in structure result in loss of activity.*

Halogenated, short-chain aliphatic acids are known to have effective herbicidal properties. Examples include sodium trichloroacetate and dalapon (sodium 2,2-dichloropropionate) (*1*).

Unusual plant-growth regulating properties of *cis*-3-chloroacrylic acid and its salts have been discovered. Sodium *cis*-3-chloroacrylate (*2*) is an efficient cotton defoliant (*3*) and crop